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Effect of formic acid exposure on keratin fiber derived from poultry feather biomass

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Abstract

Converting poultry feather biomass into useful products presents a new avenue of utilization of agricultural waste material. However, not much is understood about the poultry feather structure or methods to process it. In this study, formic acid vapor is systematically allowed to penetrate the feather fiber structure, which is composed of keratin. The diffusion kinetics show Fickian behavior during absorption. After very long times, i.e., greater than 10^3 h, the absorption experiments are stopped and the formic acid is allowed to desorb from the keratin material. The desorption kinetics of formic acid out of the keratin fiber do not mirror the absorption kinetics, indicating a change in the keratin microstructure. DSC and NMR spectroscopy analyses on the keratin fiber show a reduction in the area of the crystalline melting peak and solubilization of amino acids upon formic acid exposure. This indicates that the crystallinity is disrupted resulting in more amorphous fraction in the keratin polymer.

Keywords: Diffusion; Keratin; Fiber; Crystallinity; Poultry feather; Biomass

1. Introduction

The US poultry industry produces about 4 billion pounds of waste feathers each year (Parkinson, 1998). The feathers present a problem for the poultry industry. Currently, the feathers are autoclaved into a low nutritional value animal feed. At best, the poultry producers make a marginal profit on this animal feed. In some locations, regulatory prohibitions do not allow the feather waste to be used as feed. In these cases, feather disposal is by burial. An improved, more effective, and hopefully profitable utilization of the poultry feather waste is desirable.

A process developed by the USDA (Gassner et al., 1998) allows for the efficient cleaning of the feathers and separation into a useable feather fiber fraction. Keratin is a hierarchical structure, consisting of sub-nanometer-sized amino acids that polymerize in a known sequence into a large molecular weight protein molecule that is on the order of 10^1–10^2 nm in size. Feather keratin protein has a molecular weight of approximately 10,500 and a cysteine/cystine content in the amino acid sequence of 7% allowing for sulfur–sulfur bonding or cross-linking in the keratin (Arai et al., 1983). According to the amino acid sequence, keratin has about 40% hydrophilic chemical groups and 60% hydrophobic chemical groups in its structure. The protein molecules can then assemble into an α-helix, a β-sheet, or a random coil macrostructure (Schmidt and Line, 1996). Keratin feather fiber is 41% α-helix, 38% β-sheet, and 21% random (disordered) structures. The α-helical structure contains intra-molecular hydrogen bonds between the amide and carbonyl groups in the protein backbone. The β-sheet structure contains inter-chain hydrogen bonding between the amide and carbonyl groups in the protein backbone. The hydrogen bonding can be correlated with the bound water in the

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protein structure (Schmidt and Jayasundera, 2003). The helices can pack together to form crystals. The semi-crystalline and cross-linked structure in keratin feather fiber results in a polymer with a relatively high elastic modulus of approximately 3.4–5 GPa (Fraser and MacRae, 1980). A keratin feather fiber obtained from poultry feather is shown in Fig. 1.

The keratin feather fiber has many possible uses including a short-fiber reinforcement for polymers (Barone and Schmidt, 2005). The keratin feather fiber can be a fiber source for the woven, non-woven, and paper industries (Schmidt and Line, 1996). The fiber can easily be made into filters, paper, and insulation material without altering the traditional processes used to make these products. The keratin from the poultry feathers can be reduced and then solubilized so that it can be used in cosmetics or pressed into films for biodegradable coatings or for cell culturing substrates (Schrooyen et al., 2000; Yamauchi et al., 1998). However, reduction techniques are multi-step, long time chemical processes. A simpler method of solubilization would therefore be advantageous and offer easier processing methods as well as new industrial opportunities for the feather biomass.

It is known that some polyamides will dissolve in formic acid at room temperature (Reddy et al., 2002; Sneshkoff et al., 2002). Polyamides, also known as "nylons" are semi-crystalline polymers with a fair amount of hydrogen bonding. Keratin is a biologically formed polyamide that is also semi-crystalline with a fair amount of hydrogen bonding. It is therefore possible that formic acid also has a similar effect on the keratin feather fiber, i.e., the amorphous and crystalline fractions will dissolve in formic acid. If so, this will provide an easily processable form of keratin. Formic acid is used to "descale" wool keratin (Pielesz et al., 2003), which removes part of the outer layer of the wool fiber.

Fig. 1. Scanning electron micrograph of a keratin feather fiber. Magnification is 2500×.

Wool keratin has a much more complex morphology than feather keratin.

In this paper, the effect of formic acid on keratin feather fiber obtained from poultry feather biomass is monitored. Formic acid vapor is systematically allowed to penetrate the keratin structure as opposed to simply dissolving the fiber in formic acid. Although this process is slower, the diffusion kinetics of the formic acid into the keratin can be quantified and the step-by-step effect of the formic acid on the keratin structure measured. Formic acid is a small, flat organic molecule and the largest dimension of the molecule is approximately 0.3 nm as determined from molecular mechanics calculations (*Alchemy* from Tripos, St. Louis, MO). It is expected that formic acid can diffuse through a variety of media because of its size. The rate of diffusion of formic acid into keratin feather fiber of different sizes is determined. The desorption kinetics are subsequently measured and evaluated. The effect of formic acid on the keratin structure is characterized using differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) spectroscopy.

2. Experimental

2.1. Keratin feather fiber

Keratin feather fiber was obtained from Featherfiber® Corporation (Nixa, MO). The keratin feather fiber was cleaned and separated from the quill fraction according to a process developed and patented by the USDA (Gassner et al., 1998). The feather fiber had a uniform macroscopic diameter of approximately 0.0005 cm. The density of feather fiber was determined by displacing a known volume and weight of ethanol with an equivalent amount of fiber. A density value of $\rho_f = 0.89$ g/cm^3 was obtained.

Fibers of 0.02 cm length were made by grinding feather fiber using a Retsch ZM 1000 centrifugal grinder with a 0.02 cm screen. The rotational velocity of the instrument was 15,000 rpm and contained a torque feedback so as to not feed in too much material and overload the motor. The fiber was fed in slowly to avoid motor overload and to minimize frictional heating of the instrument and the fiber.

Fibers of 0.0053 cm length were made by grinding the fiber on a Retsch PM 400 ball mill. Feather fiber was loaded into 500 ml stainless steel grinding vessels so that it occupied about a quarter of the volume. The grinding media were four 40 mm stainless steel spheres for a total of 1132 g grinding media. Grinding proceeded at 200 rpm for 90 min.

To assess the fiber size distribution, each ground fraction was sieved on a Retsch AS 2000 vibratory mill. For the longer fiber lengths, 10 mm glass beads were used as

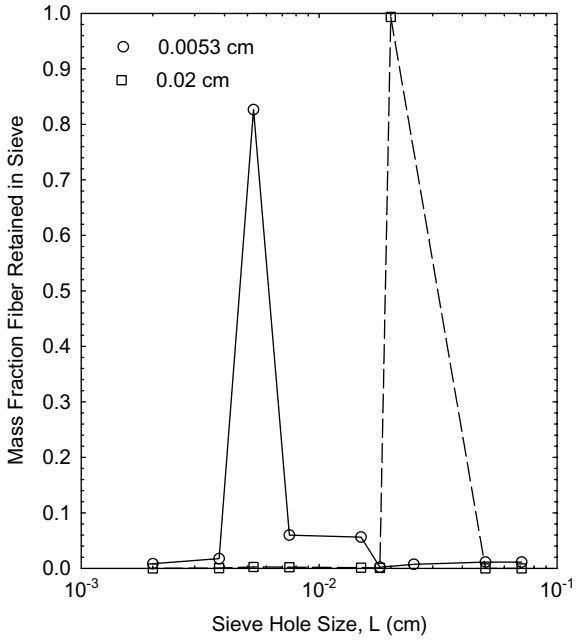

Fig. 2. Results of sieving analysis of ground feather fiber showing mass fraction retained in sieve versus sieve hole size.

sieving aids to aid the separation process. Sieving occurred at a constant frequency concurrent with the electrical frequency but amplitude and time could be varied. The material was loaded into the top sieve of the stack. The sieving stack contained eight sieves with hole sizes from 0.0710 to 0.0038 cm. Sieving proceeded at an amplitude of 1.0 (arbitrary instrument scale) for 60 min. The size distribution for each average fiber size is shown in Fig. 2. The size distribution was obtained by weighing the amount of fiber remaining in each sieve after sieving and dividing by total fiber mass to obtain the y-axis and plotting against the sieve hole size, which determined the fiber length, L. Henceforth, experiments refer to the average fiber sizes found, which were the dominant mass fractions of fiber retained at each sieving condition, 0.0053 and 0.02 cm for the ball mill and centrifugal mill, respectively.

2.2. Diffusion experiments

A small amount of each size fiber was weighed and placed in a 4 ml vial. The feather fiber weight was the diffusing media weight, w_0. The feather fibers used were the fractions obtained from grinding, i.e., different sizes were not separated out and used. For the 0.02 cm fibers, the starting feather fiber weight was $w_0 = 0.1410$ g. For the 0.0053 cm fibers, the starting feather fiber weight was $w_0 = 0.8005$ g. This uncovered 4 ml vial of fiber was subsequently placed in a 20 ml vial filled with 9 ml of 96 vol% formic acid. This larger vial was then covered to allow for the 20 ml container to fill with formic acid vapor. The 4 ml vial of fiber was removed periodically, the outer surface wiped clean, and weighed. The mass gain of the feather fiber was monitored as a function of time. The mass gain of feather fiber represented the diffusion of formic acid into the fiber. The absorption of formic acid was defined by

$$\text{absorption} = \frac{m_t}{w_0}, \tag{1}$$

where $m_t = m_a - w_0$. The term m_a was the total mass of the formic acid/feather fiber system, i.e., the initial feather fiber mass plus the formic acid absorbed. Consequently, the initial feather fiber mass was subtracted off to estimate m_t, the amount of formic acid that had entered the feather fiber at time, t. Eq. (1) shows the percentage of formic acid absorbed relative to the original amount of fiber. The starting volume of 9 ml of 96 vol% formic acid was equivalent to 10.89 g of 96 vol% formic acid. There was minimal loss of formic acid, i.e., the mass of formic acid left in the 20 ml vial and the mass of formic acid absorbed by the keratin fiber was within 10.89 g by, at most, 7%. The most likely loss of formic acid was probably due to some loss when weighing the residual formic acid in the 20 ml vial. Desorption experiments were performed in the same way by monitoring the mass loss of formic acid from the keratin feather fiber as a function of time. The same equations were used. All experiments were performed at room temperature, which was 21 °C. Four experiments were run with the 0.0053 cm diameter feather fiber and the standard deviation of the diffusion results was 2.6%, which, graphically, was within the size of the symbols used in the figures.

2.3. DSC experiments

The thermal transitions were characterized using a TA Instruments D910S Differential Scanning Calorimeter (DSC) in a nitrogen atmosphere. For each experiment, the temperature was equilibrated at 20 °C for several minutes before heating. The heating rate was kept constant at 10 °C/min until 300 °C. DSC experiments were performed before formic acid absorption, during formic acid absorption, and after formic acid desorption. Only one temperature scan was used to retain the integrity of the protein.

2.4. NMR experiments

To check the solubility of keratin feather fiber in formic acid, a 0.5 cm inner diameter NMR tube was filled with 0.09 g of 0.1 cm long fiber and then with 0.6 ml of 65 wt% formic acid/D_2O solution. This made for a 0.17 vol% feather fiber solution. The formic acid was less concentrated because of the D_2O for the NMR experiments. The glass NMR tubes were fire-sealed to minimize atmospheric interference and stored at 50 °C.

NMR analysis was performed on the solution periodically and the peak associated with the amino acid cysteine was monitored.

3. Results and discussion

3.1. Keratin feather fiber structure

The DSC curves for 0.02 cm keratin feather fiber before formic acid treatment are shown in Fig. 3. A large, low-temperature peak was observed at 95 °C. This peak depicted the amount of bound water in the keratin structure. This peak is sometimes referred to as the "denaturation" temperature. A second peak was observed at 241 °C and was the crystalline melting peak (Schmidt and Line, 1996). The 0.0053 cm keratin feather fiber exhibited qualitatively similar behavior although the effect of grinding seemed to shift the crystalline melting peak slightly. The DSC curve for 0.0053 cm keratin feather fiber was not shown for clarity. The "noise" in all of the curves at temperatures above the melting temperature corresponded to breaking of the cross-links and concurrent degradation of the keratin fiber. The degradation was observed as a color change from white to black.

The observed peaks appeared consistent with the physical model for keratin suggested by Feughelman (1997). This model proposed two types of keratin "structure", a semi-crystalline network and a cross-linked network. The bound water formed a third hydrogen-bound structure inter-twined with the keratin. The bound water network can be changed by drying the keratin feather fiber in a vacuum oven. This results in a significant decrease in the low temperature peak area, i.e., drying denatures the protein. Attempts to dry keratin feather fiber at 110–160 °C over several weeks in a vacuum oven resulted only in a reduction of the low temperature peak to approximately a third of its original area. The low temperature peak was eliminated completely only after drying in the DSC under nitrogen at temperatures of 110–160 °C for several minutes. This indicated that there was perhaps rapid re-hydration of the keratin upon removal from the vacuum oven and through the DSC sample preparation process. Medley (1966) reported diffusion coefficients of water in dry and wet (saturated) keratin of 7.5×10^{-9} cm^2/s and 2.0×10^{-7} cm^2/s, respectively. For the 0.0005 cm diameter fibers used, this would correspond to diffusion times of 33 s for dry keratin and 1.25 s for wet keratin. So, keratin could re-saturate with water rather quickly after drying. The diffusion data from Medley suggested that water associated quickly with water already present in the keratin structure and diffusion into the protein could happen quickly and easily.

3.2. Diffusion of formic acid into keratin feather fiber

The absorption of 96 vol% formic acid into the 0.0053 and 0.02 cm keratin feather fiber plotted on a linear scale is shown in Fig. 4a. In Fig. 4a, it is observed that there was rapid absorption of formic acid, which is typical of many absorption experiments (see for instance Banerjee et al., 1995; Lee et al., 1999; Malveau et al., 2001). Typically, the material saturates after a certain amount of time. However, after about 400 h, the keratin did not appear to saturate but instead there was slowing down of the absorption kinetics. For comparison purposes, each set of absorption data was normalized by the maximum value, such that (m_t/w_0)/

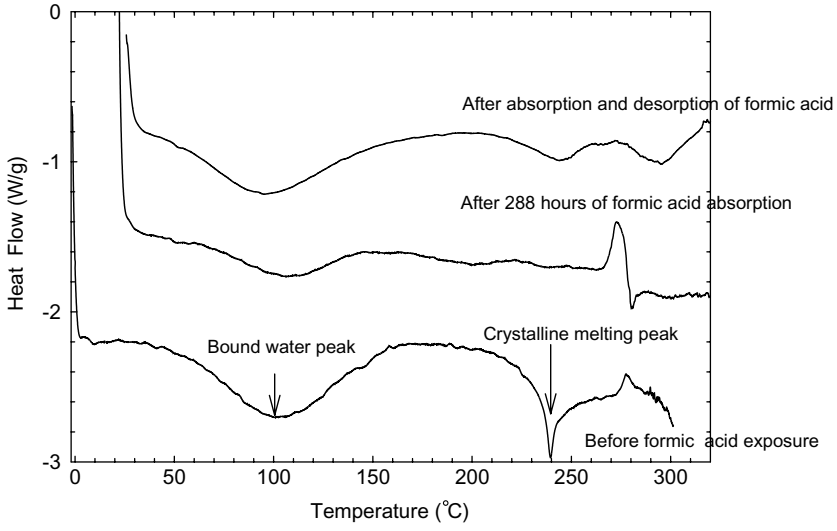

Fig. 3. DSC curves of 0.02 cm keratin feather fiber showing denaturation peak around 95 °C and crystalline melting peak around 240 °C.

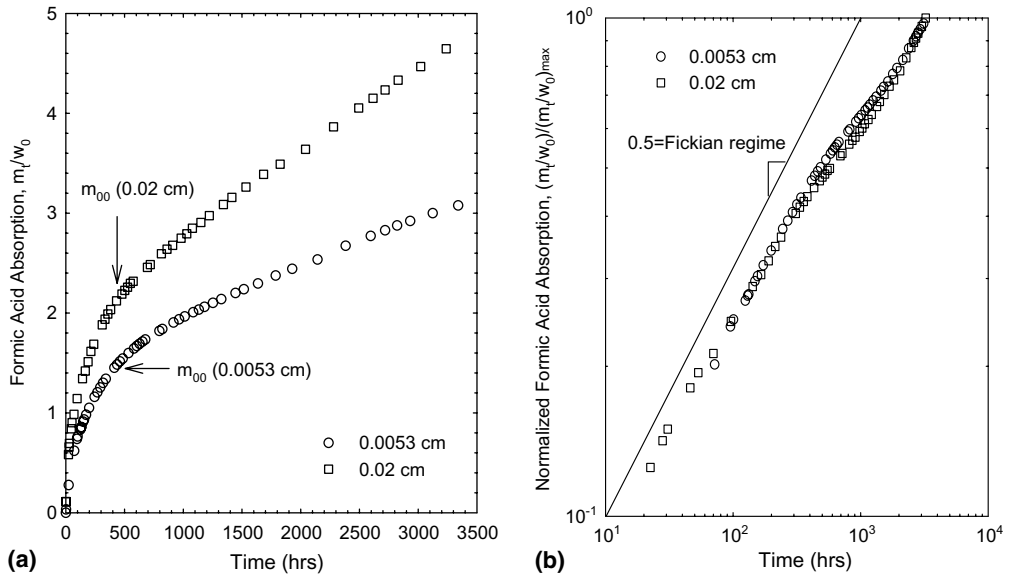

Fig. 4. (a) Linear plot of absorption kinetics of 96 vol% formic acid into keratin feather fiber; (b) log–log plot of normalized absorption kinetics for comparison.

$(m_t/w_0)_{max}$ is plotted as a function of time on a log–log scale in Fig. 4b. Fickian kinetics were realized when the mass gain or mass loss scaled with time in the following manner: $\log[(m_t/w_0)/(m_t/w_0)_{max}] \sim (\log t)^{0.5}$ (Crank, 1964). Fickian kinetics were observed before and after the inflection point at 400 h. After 3300 h of absorption (138 days) the keratin feather fiber had still not saturated with formic acid. Although each size of feather fiber attained a different magnitude of formic acid absorption as shown in Fig. 4a, the normalized data collapse on one another in Fig. 4b showing that the kinetics of formic acid absorption into keratin feather fiber were independent of the size of the keratin particles.

After a few days of formic acid absorption, keratin feather fiber turned a reddish-pink color that changed to a brownish color at long formic acid exposure times. Although the origin of the color change was not investigated, a chemical reaction between the formic acid and amino acids in the keratin structure could have occurred. The inflection and the color change develop at about the same time. Some of the keratin feather fiber was removed at 288 h, which was about the time the keratin feather fiber turned from red to brown. The keratin fiber was allowed to sit at ambient conditions for a day and then a DSC performed. It was found that the crystallinity had disappeared from the keratin structure and this result was also plotted in Fig. 3.

The absorption rate in 1/h was found from the slope of the m_t/w_0 versus time curve or $m_t/(t \times w_0)$. The absorption rates are plotted in Fig. 5. It was found that the absorption rate was not constant, but decreased with time. This suggested a non-steady state process. In fact, as absorption proceeds, the formic acid occupied more space in the keratin feather fiber structure and there was less space remaining for additional formic acid molecules to occupy.

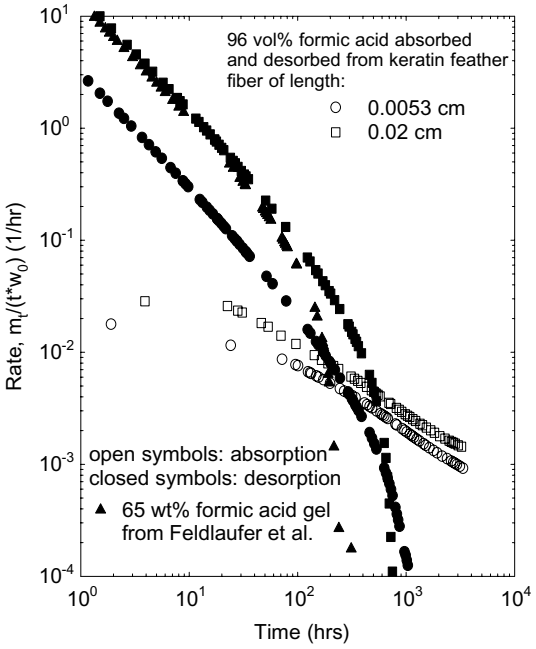

Fig. 5. Absorption and desorption rates of 96 vol% formic acid from keratin feather fiber and 65 wt% formic acid from gel.

By making some assumptions, it was possible to find diffusion coefficients from the absorption kinetics shown in Fig. 4. Fick's Second Law applied based on the observed scaling of absorption with time and the presence of non-steady state kinetics (Crank, 1964)

$$\frac{\delta c}{\delta t} = D\frac{\delta^2 c}{\delta x^2} \neq 0, \tag{2}$$

where c is the concentration of diffusing species, x is the diffusing distance, and D is the diffusion coefficient. Fick's Second Law said that the concentration of diffusing species was changing with time. The solution for Eq. (2) for various boundary conditions is given by Crank (1964) but can be simplified in the limit of low absorption time by

$$\frac{m_t}{m_\infty} \approx 4\left(\frac{Dt}{\pi r^2}\right)^{1/2} \tag{3}$$

for a cylindrical geometry (fiber) where r is the diffusing distance and is represented here as the radius of a keratin feather fiber, $r = 0.0005$ cm. The term m_∞ is the weight of formic acid inside the feather fiber at saturation, i.e., when absorption ceases. It was assumed that diffusion proceeded from the surface of the fiber (largest surface area) into the fiber bulk with negligible diffusion into the fiber ends (smallest surface area). Eq. (3) applied over the range $0 < (m_t/m_\infty) < 0.6$ for a plane sheet (Liu, 2003). Crank (1964) noted the time range over which Eq. (3) is valid may be much lower for a cylinder than for the plane sheet case. Eq. (3) was "approximate" because the solution to the diffusion equation for a cylinder was quite complex and there was no exact form.

Saturation was not observed in the time frame of the performed absorption experiments, i.e., the keratin feather fiber could additionally absorb formic acid for months. However, the inflection observed in Fig. 4 may give insight into a saturation event. First, the feather fiber turned a brownish color during the inflection or non-Fickian absorption regime. The feather fiber turned the same color if formic acid was simply poured into a bottle full of feather fiber. Consequently, the inflection observed in the absorption kinetics might have been the saturation of the internal structure of the fiber with formic acid and the subsequent existence of formic acid on the surface of the fibers, in the void space between packed fibers. In this case, m_∞ would be the value at approximately 400 h because it corresponded to the saturation of keratin feather fiber, not the feather fiber agglomerate.

Another possible explanation for the inflection observed in the absorption kinetics might be that a structural change in the keratin had occurred and the inflection represented the adjustment of the new keratin structure to the formic acid absorption. In this case, it seemed appropriate to use the mass value at approximately 400 h as the saturation value, m_∞, because it represented the saturation of native, or unchanged, keratin feather fiber.

It was assumed that m_∞ could be represented by the amount of formic acid absorbed at approximately 400 h because it either represented the equilibrium formic acid value of native (unchanged) keratin fiber or of formic acid residing inside the fiber. In the diffusion studies referenced above, some of the absorption curves flattened out over time while others slowed down to a very low slope. The current case seemed to be more closely related to the latter where the "saturation" event was a severe slope change. The values of m_∞ for 0.02 and 0.0053 cm keratin fiber were 0.3140 and 1.1622 g, respectively. This resulted in a diffusion coefficient of $D = 1.28 \times 10^{-10}$ cm^2/h (3.56×10^{-14} cm^2/s) for the 0.02 cm keratin feather fiber and a diffusion coefficient of 1.27×10^{-10} cm^2/h (3.54×10^{-14} cm^2/s) for the 0.0053 cm keratin feather fiber for absorption up to 400 h. The diffusion coefficients are plotted in Fig. 6 for the absorption of formic acid into keratin feather fiber. Also plotted in Fig. 6 are diffusion coefficients that utilize m_f, the final mass of formic acid measured after 138 days. The measured diffusion coefficients were about five orders of magnitude slower than those measured by Medley (1966) for water in dry keratin.

The NMR experiments exhibited a definitive structural change in the keratin fiber when it was exposed to formic acid over time. At the start of the experiment, no peaks appeared in the spectra but some started to develop after about 2 weeks, which was concurrent with the 400 h inflection. Fig. 7 shows the evolution of the feather keratin NMR spectrum over 39 weeks of formic acid exposure. Slowly, the keratin dissolved in the diluted formic acid and the solution changed color from clear to a dark brown color over 39 weeks at 50 °C. The feather fiber visibly began to disappear as the color changed. Cystine CH_2–S–S– bonds, or cross-links,

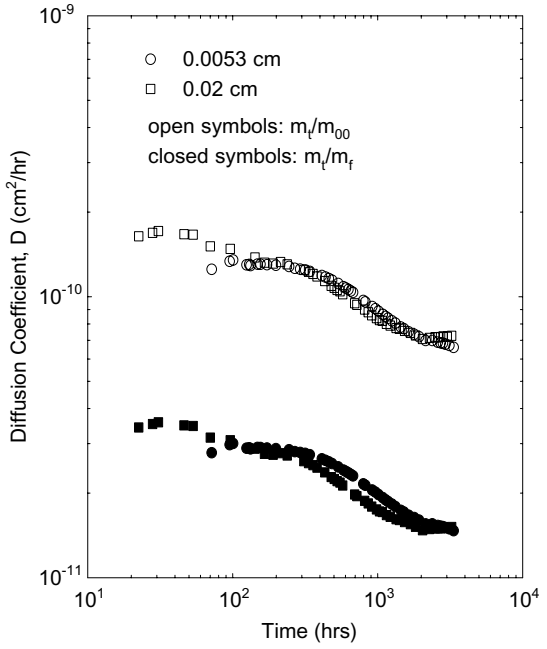

Fig. 6. Diffusion coefficients of 96 vol% formic acid into keratin feather fiber obtained from absorption experiments.

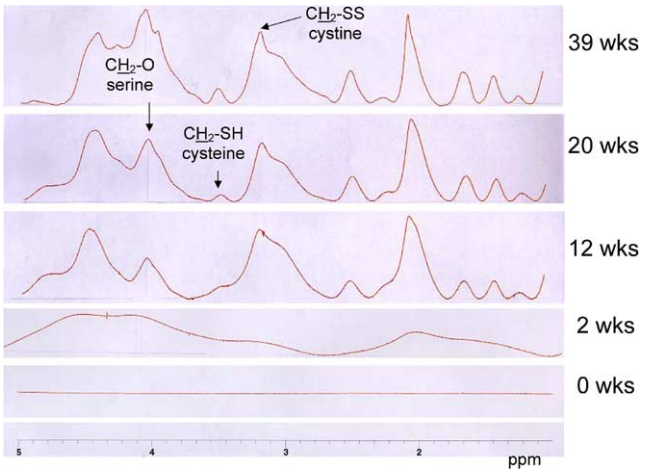

Fig. 7. NMR analysis showing solubility of keratin feather fiber in 65 wt% formic acid as a function of time.

under redox conditions form cysteine, CH_2–S–H, bonds. Cysteine appeared at 3.5 ppm in the NMR spectrum and cysteine at 3.2 ppm. It is clear that cross-links were broken in the keratin structure. Many of the amino acids solubilize between 12 and 20 weeks because the peaks remained relatively unchanged after this time. However, the serine peak at 4.0 ppm and the cysteine and cysteine peaks changed more slowly. Although the formic acid solution used in the NMR experiments was more dilute, similar conclusions could be made about the keratin structure. Although the diffusion of formic acid into the keratin appeared slow, it was a first attempt at altering the structure of the keratin to obtain an amorphous fraction for further processing. It is clear from the NMR spectrum evolution that amino acids in feather keratin were dissolving.

The diffusion occurred into an agglomerate of feather fiber. The average size of the fiber was varied to note the effect of particle surface area on diffusion. In addition, agglomerates of different sized particles would be expected to have different bulk densities and therefore different void volumes in the agglomerate. A simple mathematical analysis can show if the formic acid was diffusing into the keratin fibers (absorption) or residing on the surface of the fibers and in void spaces between fibers in the agglomerate (adsorption). For instance, the total fiber surface area of each agglomerate, S_T, was found from the data in Fig. 2 and the w_0 values. The surface area of a fiber of length L and radius r is $S = 2\pi r^2 + 2\pi rL$. The values of L were those depicted in Fig. 2. The total surface area of fibers in the vial was $S_T = S \times N$, where N was the total number of fibers in the vial. The total number of fibers in the vial was $N = v'/v_f$ where $v' = w_f w_0/\rho_f$ was the volume fraction of each fiber in the vial and $v_f = \pi r^2 L$ was the actual volume of a fiber of each size in the vial. In this scheme, w_f was the weight fraction of each size fiber and w_0 was the actual starting weight of fiber. The value w_f was also found from Fig. 2 and ρ_f was 0.89 g/cm^3, the density of feather keratin fiber. This simple estimate relies on ratios, which cancels out any corrections to the fiber surface area due to roughness. The ratio of the total fiber surface areas was $S_T(0.0053\text{ cm})/S_T(0.02\text{ cm}) = 5.85$. By contrast, the ratio of the surface area, S, of one fiber of each length would be $S(0.0053\text{ cm})/S(0.02\text{ cm}) = 0.283$. The ratio of initial fiber mass used was $w_0(0.0053\text{ cm})/w_0(0.02\text{ cm}) = 4.57$. The bulk density, ρ_b, of each fiber agglomerate at the start of the experiment was determined by measuring the volume of fiber in the 4 ml vial and then dividing w_0 by that volume to get $\rho_b(0.0053\text{ cm}) = 0.202$ g/cm^3 and $\rho_b(0.02\text{ cm}) = 0.062$ g/cm^3 for a ratio of $\rho_b(0.0053\text{ cm})/\rho_b(0.02\text{ cm}) = 3.26$. The void volume of each fiber agglomerate can be found from the equation $\rho_b = w_0/(v_{solids} + v_{voids})$. The volume of solids was simply $v_{solids} = w_0/\rho_f$. The void volume ratio was then $v_{void}(0.0053\text{ cm})/v_{void}(0.02\text{ cm}) = 1.16$.

For comparison, the ratio of the weight of formic acid absorbed at "saturation" ($t = 400$ h) was $m_\infty(0.0053\text{ cm})/m_\infty(0.02\text{ cm}) = 3.88$ and the ratio of the weight of formic acid absorbed at the end of the experiment ($t = 3300$ h) was $m_f(0.0053\text{ cm})/m_f(0.02\text{ cm}) = 3.76$. So it appeared as if the amount of formic acid absorbed by the keratin feather fiber depended on the amount of feather fiber and not on the void volume in the agglomerate. In other words, the concurrence of the diffusion coefficients for each size of fiber and the concurrence of the ratio of formic acid absorbed (3.88 and 3.76) with the amount of feather fiber present (4.57) indicate that the formic acid resided inside the fiber and not on the outside of the fibers in the void space of the agglomerate. The amount of formic acid absorbed was several orders of magnitude higher than the volume that would represent a monolayer of formic acid that would be adsorbed on the keratin fiber surface. The volume of a monolayer of formic acid was found from the largest dimension in the formic acid molecule, which was 0.3 nm as determined from molecular mechanics calculations, and the density of formic acid, 1.22 g/cm^3 and was approximately 3×10^{-5} cm^3. In addition, the observed normalized absorption kinetics were similar indicating that absorption rate was independent of fiber size. However, the absolute magnitude of formic acid absorbed does depend on the fiber size and amount of fiber.

The keratin feather fiber structure was 41% α-helix, 38% β-sheet, with the balance being disordered protein structures (Schmidt and Jayasundera, 2003). The keratin feather fiber was predominantly α-helix structure and the distance between successive twists was about 0.3 nm. This dimension was approximately the size of the largest dimension of the formic acid molecule in three-dimensional space. Therefore, it was possible for the formic acid to physically enter the keratin helix.

Water had a smaller dimension than 0.3 nm so it was easily accepted because of the large percentage of hydrophilic sites and may explain the faster diffusion coefficients observed by Medley (1966).

3.3. Diffusion of formic acid out of keratin feather fiber

The desorption data for the formic acid from the keratin feather fiber samples is plotted in Fig. 8. By accident, 1.5 g of the liquid formic acid spilled into the 0.2 cm keratin feather fiber sample at the end of the absorption experiment and before the desorption experiment. This explained why the normalized desorption data in Fig. 8 did not collapse like in Fig. 4b. In fact, the 0.2 cm feather fiber desorption data was faster because the formic acid did not have time to enter the keratin structure and just desorbed from the surface. For either size, all of the formic acid absorbed had desorbed in about a third of the time it took to absorb. The desorption rates were found in the same manner as the absorption rates and are also plotted in Fig. 5. Desorption of formic acid from keratin feather fiber was faster than absorption of keratin feather fiber as observed in Fig. 8.

The fact that the desorption kinetics did not mirror the absorption kinetics indicated that a structural change may have occurred to the keratin upon the introduction of the formic acid into the protein structure. This was indeed confirmed using DSC and NMR experiments. The formic acid dissolved the keratin resulting in a change in the state of crystallinity. After formic acid desorption, the crystalline peak had re-appeared but was distinctly less pronounced. The presence of more amorphous fraction would explain why desorption was faster than absorption, since diffusion of gas molecules was through the amorphous fraction of polymers (Vieth, 1991) with the crystals acting as impediments to diffusion. The lack of crystallinity would indicate an easier diffusion path.

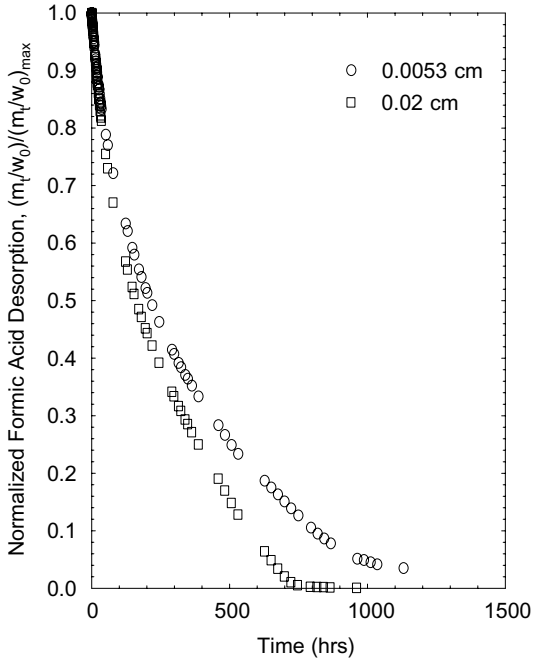

Fig. 8. Normalized linear plot of desorption kinetics of 96 vol% formic acid from keratin feather fiber.

Pielesz et al. (2003) used FTIR spectroscopy to show that formic acid treatment of wool keratin increased the amount of β-sheet and disordered structures. The amount of crystallinity was related to the amount of α-helical keratin. So the DSC and NMR results shown here appeared consistent with the FTIR results of Pielesz et al. Increase of β-sheet and disordered structure resulted in less crystallinity in both wool and feather keratin.

The lack of crystallinity in the presence of formic acid implied that the keratin can be processed into various geometries because mobile amorphous polymer molecules remain. For instance, upon desorption, what was left in the vial was a hard keratin cylinder. The cylinder was completely cohesive and the vial must be broken to remove the hard cylinder. Following this observation a simple experiment was performed by preparing 6.6 wt% solutions of feather fiber in 96% formic acid allowing them to dissolve for 2 days. A clear, pink liquid forms on the surface of the vial after two days. The liquid was sampled and cast onto a glass plate. A thin film remains. Simply dissolving in formic acid was a method to prepare cohesive keratin objects without multi-step reduction processes. The crystallinity was disrupted during solubilization and re-appeared after the solvent was gone. All of the experiments were performed at room temperature but it is possible that raising the temperature during solubilization and film casting or perhaps using one of the popular polymer processing techniques on a blend of formic acid and keratin feather fiber may produce a formed product much faster.

Sneshkoff et al. (2002) and Reddy et al. (2002) showed that nylon dissolved in formic acid along with amino acids will form strongly hydrogen bonded networks of nylon with amino acid molecules incorporated into the structure. Upon removal of the amino acid molecules with a suitable solvent, a film was left that was called ‘‘molecularly imprinted’’. The film could then recognize only the amino acid that it once contained if that amino acid was reintroduced. This was a way to recognize certain molecules to mimic certain biological processes. It is possible that the feather keratin could be used in a similar manner that the nylon was and the keratin would be biologically derived. However, it was observed by Sneshkoff et al. that amino acids degrade in formic acid after a few days. This may be the origin of the color change observed for the feather keratin after formic acid exposure as previously noted. The ability to dissolve keratin in formic acid easily may provide a method to make polymeric products from keratin such

as natural films for biodegradable packaging or biocompatible and bioresorbable drug delivery devices, assuming the formic acid could be removed easily.

The ability of the keratin fiber to absorb and retain formic acid may have another use besides the ability to form keratin products. Formic acid has recently found a use as a pesticide in controlling parasitic mites of honeybees (Calderone, 2000; Feldlaufer et al., 1997). However, packaging and delivery of the formic acid was problematic. The keratin feather fiber may provide a safe, environmentally–friendly method to overcome this. Plotted in Fig. 5 for comparison is the desorption of diluted formic acid from the gel used by Feldlaufer et al. (1997). The desorption of 96 vol% formic acid from the keratin feather fiber compared well with the desorption of 65 wt% formic acid from the gel. Ironically, the accidental spillage of formic acid into the 0.02 cm feather fiber container showed that this formula had a similar desorption profile to the gel. This indicated that the volume of formic acid needed to match the gel was not high enough from the saturated fibers used here. To match the gel, some of the formic acid would have to reside outside the fiber surface, or larger fibers would have to be used to create more free volume for formic acid storage. Feldlaufer et al. achieved a 70% mortality rate of *Varroa* mites using the gel. The formic acid from the feather fiber was more concentrated but the release kinetics were similar indicating that the feather fiber delivery system could be at least as effective as the gel.

4. Conclusions

The diffusion of formic acid vapor into and out of agglomerates of keratin fibers obtained from chicken feathers was described. It was found that the amount of formic acid absorbed by the keratin fiber correlated with the amount of fiber in the agglomerate and not the amount of void space in the agglomerate. Diffusion of formic acid into the keratin fiber resulted in a structural change as evidenced by the reduction in the crystalline melting peak as determined by DSC and a slow increase in the intensity of amino acid peaks, specifically, the serine peak at 4.0 ppm and cysteine/cystine peaks at 3.5 and 3.2 ppm in NMR solubility studies. Subsequent desorption of formic acid from the keratin fiber agglomerates proceeded at faster rates than absorption, indicating a structural change to the keratin. Keratin can therefore be solubilized in formic acid and suitable products formed after the solvent is driven off. It was shown that the keratin feather fiber may be a suitable delivery system for formic acid. Understanding of the structure of the feather keratin is the first step in developing products from the abundance of feather biomass produced in the US each year.

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